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Product Information

Visfatin EIA Kit
for serum, plasma, culture supernatant, and cell lysates

Catalog Number **RAB0377**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
The Visfatin Enzyme Immunoassay (EIA) Kit is an *in vitro* quantitative assay for detecting visfatin peptide based on the principle of competitive enzyme immunoassay. In this assay, a biotinylated visfatin peptide is spiked into the samples and standards. The samples and standards are then added to the plate, where the biotinylated visfatin peptide competes with endogenous (unlabeled) visfatin for binding to the anti-visfatin antibody. After a wash step, any bound biotinylated visfatin then interacts with horseradish peroxidase (HRP)-streptavidin, which catalyzes a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of captured biotinylated visfatin peptide and inversely proportional to the amount of endogenous visfatin in the standard or samples. A standard curve of known concentration of visfatin peptide can be established and the concentration of visfatin peptide in the samples can be calculated accordingly.

Components
1. 96-well plate coated with secondary antibody (Item A) - RAB0377A: 96 wells (12 strips × 8 wells) coated with secondary antibody.
2. 20x Wash Buffer (Item B) - RABWASH3: 25 mL.
3. EIA Visfatin Peptide standard, Lyophilized (Item C) - RAB0377C: 2 vials.
4. Anti-Visfatin Detection Antibody, Lyophilized (Item N) - RAB0377F: 2 vials.
5. EIA Visfatin 5x Assay Diluent B (Item E) – RABDIL10: 15 mL of 5x concentrated buffer. Diluent for both standards and samples including serum, plasma, cell culture media, or other sample types.
6. Biotinylated Visfatin Peptide, Lyophilized (Item F) - RAB0377G: 2 vials.
7. HRP-streptavidin (Item G) - RABHRP3: 600 µL of 100x concentrated HRP-conjugated Streptavidin.
8. Visfatin Positive Control Sample, Lyophilized (Item M) - RAB0377K: 1 vial.
9. TMB Substrate solution (Item H) - RABTMB2: 12 mL of 3,3′,5,5′- tetramethylbenzidine (TMB) in buffered solution.
10. Stop Solution (Item I) – RABSTOP3: 8 mL of 0.2 M sulfuric acid.

Reagents and Equipment Required but Not Provided.
1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 µL to 1 mL volumes
3. Adjustable 1-25 mL pipettes for reagent preparation
4. 100 mL and 1 liter graduated cylinders
5. Absorbent paper
6. Distilled or deionized water
7. SigmaPlot software (or other software which can perform four-parameter logistic regression models)
8. Tubes to prepare standard or sample dilutions
9. Orbital shaker
10. Aluminum foil

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
For standard, positive control and sample dilutions refer to steps 6, 7, and 10 of Preparation.

1. Keep kit reagents on ice during reagent preparation steps. Equilibrate plate to room temperature before opening the sealed pouch.

2. 5x Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water.

3. Briefly centrifuge the anti-visfatin antibody vial (Item N) and reconstitute with 55 µL of Assay Diluent B to prepare the antibody concentrate. Pipette up and down to mix gently.

4. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent B. This is the anti-visfatin antibody working solution, which will be used in Procedure, step 2.

Note: The following steps may be done during the antibody incubation procedure (Procedure, Step 2).

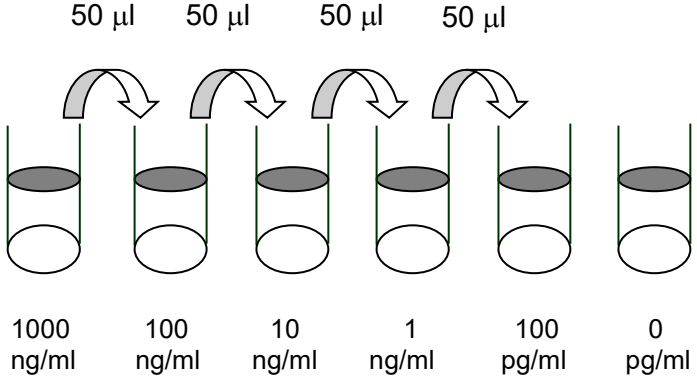
5. Briefly centrifuge the vial of biotinylated visfatin peptide (Item F) and reconstitute with 20 µL of water before use. Transfer the entire contents of the Item F vial into a tube containing 10 mL of 1x Assay Diluent B. Pipette up and down to mix gently. The final concentration of biotinylated visfatin will be 20 ng/mL.
   a. Second Dilution of Item F for Standards: Add 2 mL of Working Stock Item F to 2 mL of 1x Assay Diluent B. The final concentration of biotinylated visfatin will be 10 ng/mL.
   b. Second Dilution of Item F for Positive Control: Add 100 µL of Working Stock Item F to 100 µL of the prepared Positive Control (Item M). (Preparation, step 7) The final concentration of biotinylated visfatin will be 10 ng/mL.
   c. Second Dilution of Item F for samples: Add 125 µL of Working Stock Item F to 125 µL of prepared sample (Preparation, step 10). This is a 2-fold dilution of the sample. The final concentration of biotinylated visfatin will be 10 ng/mL.

6. Preparation of Standards: Label 6 microtubes with the following concentrations: 1,000 ng/mL, 100 ng/mL, 10 ng/mL, 1 ng/mL, 100 pg/mL, and 0 pg/mL. Pipette 450 µL of biotinylated visfatin solution into each tube, except for the 1,000 ng/mL (leave this one empty).

   Note: It is very important to make sure the concentration of biotinylated visfatin is 10 ng/mL in all standards.

   a. Briefly centrifuge the vial of standard visfatin peptide (Item C) and reconstitute with 10 µL of water. In the tube labeled 1,000 ng/mL, pipette 8 µL of Item C and 792 µL of 10 ng/mL biotinylated visfatin solution (Preparation, step 5). This is the visfatin stock solution (1,000 ng/mL visfatin and 10 ng/mL biotinylated visfatin). Mix thoroughly. This solution serves as the first standard.
   b. To make the 100 ng/mL standard, pipette 50 µL of visfatin stock solution to the tube labeled 100 ng/mL. Mix thoroughly.
   c. Repeat this step with each successive concentration, preparing a dilution series (see Figure 1). Each time, use 450 µL of biotinylated visfatin and 50 µL of the prior concentration until 100 pg/mL is reached. Mix each tube thoroughly before the next transfer.
   d. The final tube (0 pg/mL visfatin and 10 ng/mL biotinylated visfatin) serves as the zero standard (or total binding).

Figure 1.
Dilution Series for Standards



7. Positive Control Preparation: Briefly centrifuge the positive control vial (Item M) and reconstitute with 100μL of water. The Positive Control is a cell culture media sample that serves as a system control to verify that the kit components are working. The resulting OD will not be used in any calculations; The Positive Control may be diluted further if desired, but be sure the final concentration of biotinylated visfatin is 10 ng/mL.

8. If Item B (20x Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer.

9. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate should be diluted 100-fold with 1x Assay Diluent B.

10. If you wish to perform a 2-fold dilution of the sample, proceed to step 4c. If you wish to perform a higher dilution of your sample, dilute the sample with Assay Diluent E before performing step 5c. Example: (to make a 4-fold dilution of sample):
    a. Dilute sample 2-fold (62.5 μL of sample plus 62.5 μL of 1X Assay Diluent B.).
    b. Perform step 4c (125 μL of working solution Item F plus 125 μL of sample prepared above).

    The total volume is 250 μL, enough for duplicate wells on the microplate. It is very important to make sure the final concentration of the biotinylated visfatin is 10 ng/mL.

    Note: Optimal sample dilution factors should be determined empirically, however, recommended dilution factors for serum: Human = 4x, Mouse = 4x, Rat = 2x.

Storage/Stability

The entire kit may be stored at –20 °C to –80 °C for up to 6 months from the date of shipment. For extended storage, it is recommended to store at –80 °C. Avoid repeated freeze-thaw cycles.

The remaining kit components may be stored at 2–8 °C.

Opened microplate strips and Item N may be stored for up to 1 month at 2–8 °C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

The kit remains active for up to 6 months.

Procedure

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.

2. Add 100 μL of anti-visfatin antibody (see Preparation, step 4) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or incubate overnight at 4 °C.

3. Discard the solution and wash wells 4 times with 1x Wash Buffer (200-300 μL each). Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 μL of each standard (see Preparation, step 6), positive control (see Preparation, step 7) and sample (see Preparation, step 10) into appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4 °C.

5. Discard the solution and wash 4 times as directed in step 3.

6. Add 100 μL of prepared HRP-Streptavidin solution (see Preparation, step 9) to each well. Incubate for 45 minutes with gentle shaking at room temperature. It is recommended that incubation time should not be shorter or longer than 45 minutes.

7. Discard the solution and wash 4 times as directed in step 3.

8. Add 100 μL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).

9. Add 50 μL of Stop Solution (Item I) to each well. Read absorbances at 450 nm immediately.

Results

Calculations

Calculate the mean absorbance for each set of duplicate standards, controls, and samples, and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

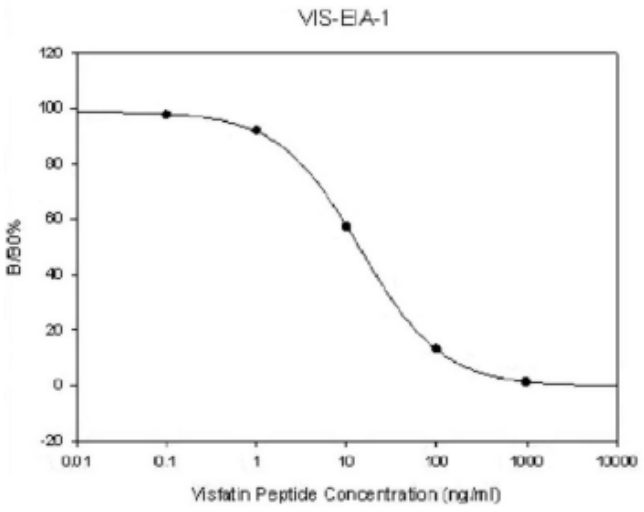
Percentage absorbance =

(B – blank OD)/(B_o – blank OD)

B = OD of sample or standard
B_o = OD of zero standard (total binding)

Typical Data

Standard curve(s) is for demonstration only. Standard curve(s) must be run with each assay.



Product Profile

Sensitivity: The minimum detectable concentration of visfatin is 0.778 ng/mL or 6.82 pM.

Detection Range

0.1-1,000 ng/mL

Reproducibility:
Intra-Assay: CV <10%
Inter-Assay: CV <15%

Specificity

Cross Reactivity: This kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, Angiotensin II, NPY, and APC.

References

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2. Abdin A. et al., Effect of propranolol on IL-10, visfatin, Hsp70, iNOS, TLR2, and survivin in amelioration of tumor progression and survival in Solid Ehrlich Carcinoma-bearing mice. Pharmacological Reports, Available online 8 August 2014.
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4. Mabrouk R. et al., Serum Visfatin, Resistin and IL-18 in A Group of Egyptian Obese Diabetic and Non Diabetic Individuals. The Egyptian Journal of Immunology, **20**(1) (2013).
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Appendix

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 2 may change to overnight
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Air bubbles in wells	Remove bubbles in wells
	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at –20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Stop solution should be added to each well before measurement.

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